ELECTROPHORESIS APPARATUS

Field Of The Invention

The present invention relates to an electrophoresis apparatus and methods of its

5   use for fractionation of a complex sample.

Background Of The Invention

All publications and patent applications herein are incorporated by reference to

the same extent as if each individual publication or patent application was specifically

and individually indicated to be incorporated by reference.

10   The following description includes information that may be useful in

understanding the present invention. It is not an admission that any of the information

provided herein is prior art or relevant to the presently claimed inventions, or that any

publication specifically or implicitly referenced is prior art.

Electrophoresis has been widely applied in separating proteins, nucleic acids, and

15   other charged molecule species for analytical or preparative purposes, and also in the

analytical or preparative fractionation of heretogeneous populations of dispersed cells or

other types of macroscopic particles. In the analysis of complex ampholytic samples,

such as in proteomics, it would often be desirable to reduce the complexity of a sample by

pre-fractionation. Two dimensional electrophoresis (2DE) is believed to be currently the

20   most commonly used separation method in proteomics. In the first dimension of 2DE,

conventional gel isoelectric focusing (CGIEF) or better yet, immobilized pH gradient IEF

(IPGIEF) are used to separate proteins according to their pI values.

Both CGIEF and IPGIEF have numerous practical problems including a limited

sample loading capacity, a limited dynamic range, precipitation of proteins during IEF

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separation (streaking) and an inability to tolerate a large amount of salts in the samples.

P.G. Righetti et al., (Electrophoresis 21, 2000, 3639-3648); P.G. Righetti et al., (Anal.

Chem. 73, 2001, 320A-326A) and D.W. Speicher et al., (Anal. Biochem., 284, 2000, 266-

278); X. Zou & D. W. Speicher, (Proteomics, 2, 2002, 58-68) have shown that pre-

5   fractionation of a complex protein sample in a multi-compartmental electrolyzer

significantly improves the performance of 2DE. It is believed that the common limitation

of both the ISOELECTRIQ2™ unit, marketed by Proteome Systems™ and the ZOOM™

unit, marketed by INVITROGEN™ is two-fold. First, the distance between the center of

the separation compartment and its walls is relatively large (greater than about 5 mm),

10   and second, the electrophoretic migration distance in each compartment is long, about 25

mm and 13 mm, respectively. The first, coupled with the fact that the separation

compartments are made of thermally insulating polymers, leads to poor Joule heat

dissipation and severely limits the electric power that can be applied to the system (max.

5 W and 3.5 W, respectively). The second, coupled with the low electrophoretic

15   mobilities brought about by the low field strength, a consequence of the limited heat

dissipation capability of the systems and the long electrophoretic migration distance,

leads to slow separation velocities. Consequently, the fractionation times in these

systems are long, 6 to 16 hours and 4 hours, respectively. Both systems use

compartments with relatively large volumes (about 5 ml and 0.7 ml for each

20   compartment, respectively), and the volume of the compartments cannot be easily

reduced.

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Gradipore Limited (Life Therapeutics) developed a small scale electrophoresis

unit for size-based and charge-sign-based fractionation of complex samples (WO

01/78878, incorporated herein by reference). It is believed that, in practice, active

cooling of at least the electrolytes was required to prevent over-heating of labile proteins

5   during electrophoresis. Gradipore subsequently developed a scaled-down version of the

GRADIFLOW™ electrophoresis unit, for size-based and charge-sign-based pre-

fractionation of complex samples. In this MICROFLOW™ system, about 3 cm x 4 cm

polymer frames, separated by polyacrylamide membranes, are stacked next to each other

to form the separation compartments and contain stagnant sample solutions. The

10   compartment stack is terminated at both ends by a large volume anode compartment and

cathode compartment. It is believed that in practice, the anolyte and catholyte are cooled

and circulated through these compartments to provide convective heat removal.

Slow separation speed of the currently known electrophoresis systems,

specifically, isoelectric fractionation systems, useful as they are, are believed to be due to

15   the failure of existing systems to sufficiently address three interrelated design limitations.

The first speed limitation comes from the fact that as the ampholytic components of a

sample approach their isoelectric state, their electrophoretic mobilities approach zero.

Consequently, when the components are close to their isoelectric state, they need an

increasingly longer time to move across a certain distance. The second speed constraint

20   comes from mechanical design problems that limit how short the electrophoretic

migration path and how small the volume of the individual compartments holding the

sample solutions can be before mechanical assembly and leak-tight sealing of the

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compartments become very difficult. The third performance limitation comes from the

amount of Joule heat that is produced during electrophoresis. Since Joule heat dissipation

occurs through the walls of the separation compartment, and since heat must first be

transported from the separation medium to the wall, both of which are inefficient

5   processes, the amount of Joule heat produced during fractionation must be limited and

external, active cooling means must be applied. This means that the electric power input

into the system to effect a separation must be limited. This results in a low electric field

strength which, in turn, results in slow electrophoretic migration velocities and

concomitant long separation times presently observed with current apparatus.

10   Accordingly, there exists a need for an electrophoresis apparatus or device

suitable for processing small volume samples while effectively dissipating the heat

generated during electrophoresis and reducing separation times. More specifically, the

second and third speed limitations discussed above can be eliminated or negated to a great

extent by selecting a structural material for the separation compartments of an

15   electrophoresis apparatus that is a good electrical insulator yet has a relatively high

thermal conductivity and specific heat. From these materials, one could make separation

compartments that act as high capacity heat sinks by creating small separation

compartments with appropriately selected characteristic dimensions in relatively large

pieces. These heat sinks would greatly mitigate the need for active external cooling

20   and/or for the reduction of the electrophoretic power used.

Summary Of The Invention

The present invention relates to an electrophoresis apparatus and methods of its

use for fractionation of a complex sample. The apparatus more specifically relates to

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Membrane-Separated Wells for Isoelectric Focusing and Trapping (MSWIFT). Primary

application areas of MSWIFT and its modes of operation are in the analytical-scale

fractionation of complex samples, such as pre-fractionation of protein samples for

proteomic analysis, preparation of fractions for mass spectral (MS) analysis, bioactivity

5   testing, enzymatic analysis, etc., rapid selection of isoelectric membranes for preparative-

scale isoelectric trapping (IET) separations, and characterization of isoelectric

membranes.

The present invention provides for an electrophoresis apparatus for characterizing,

measuring and/or altering a composition of a sample. The apparatus comprises an anode

10   and a cathode, the cathode spaced from the anode so as to define a distance along a

longitudinal axis, the anode and cathode further defining an electric field having a

direction substantially along the longitudinal axis. The apparatus includes an anode

compartment, the anode disposed therein and a cathode compartment, the cathode

disposed therein. Each of the anode compartment and the cathode compartment can be

15   configured to hold at least one electrolyte, and at least one of the anode compartment and

the cathode compartment can be configured to hold at least a portion of the sample. Each

of the anode compartment and the cathode compartment includes means for addition or

removal of a solution, a first compartment dimension, a second compartment dimension,

and a third compartment dimension. The first compartment dimension can be

20   substantially orthogonal to the direction of the electric field, and the second compartment

dimension can be substantially orthogonal to the direction of the electric field and the first

compartment dimension. A ratio of the first compartment dimension and the second

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compartment dimension defines an aspect ratio of the compartment, and the third

compartment dimension can be substantially parallel to the direction of the electric field

and substantially orthogonal to the first and second compartment dimensions. The

apparatus further comprises an ion-permeable barrier positioned between the anode

5   compartment and the cathode compartment. The ion-permeable barrier can be configured

to prevent convective mixing between compartments. At least a portion of at least one of

the anode and cathode compartments can be made of an electrically insulating material

having a thermal conductivity greater than about 1 W/mK and a specific heat greater than

about 100 J/kgK and the aspect ratio of at least one of the anode compartment and the

10   cathode compartment can be less than one.

        The electrophoresis apparatus preferably further comprises sealing means

disposed between the anode compartment and the cathode compartment. The sealing

means is preferably adapted to contain the ion-permeable barrier and provide access of

ions to the ion-permeable barrier. Wherein the sealing means is made of a water

15   insoluble polymer, the polymer can be natural or synthetic. Preferably, the water

insoluble polymer of is selected from the group consisting of polyethylene,

polypropylene, polyisobutylene, polyalkylenes, polyfluorocarbons,

poly(dimethylsiloxane), poly(dialkylsiloxane), poly(alkylarylsiloxane),

poly(diarylsiloxane), poly(ether ketones) or a combination thereof.

20   The electrophoresis apparatus further preferably comprises housing means for

containing the anode and cathode compartments. Preferably, at least a portion of the

housing means is made of a material having a thermal conductivity greater than about 1

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W/mK and a specific heat greater than about 100 J/kgK. Moreover preferably, material

of the at least portion of the housing means can be selected from the group consisting of

alumina, aluminum nitride, zirconia, zirconium nitride, boron nitride, silicon nitride,

silicon carbide, ceramics, fused silica, quartz, glass or any combination thereof.

5   The electrically insulating material of the at least one part of the anode or cathode

compartment can be preferably selected from the group consisting of alumina, aluminum

nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon carbide,

ceramics, fused silica, quartz, glass or any combination thereof.

Preferably, the ion-permeable barrier is essentially free of weekly acidic

10   functional groups or weakly basic functional groups or anionic functional groups or

cationic functional groups. Alternatively, the ion-permeable barrier can be an isoelectric

barrier.

In an alternative embodiment of an electrophoresis apparatus according to the

present invention for measuring, characterizing, or altering a composition of a sample, the

15   apparatus comprises an anode and a cathode, the cathode spaced from the anode so as to

define a distance along a longitudinal axis, the anode and cathode further defining an

electric field having a direction substantially along the longitudinal axis. The apparatus

includes an anode compartment having an anode disposed therein and a cathode

compartment having a cathode disposed therein. At least one separation compartment is

20   preferably positioned between the anode and cathode compartments. Each of the anode

compartment, cathode compartment and at least one separation compartment can be

configured to hold at least one electrolyte. At least one of the anode compartment,

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cathode compartment and at least one separation compartment can be configured to hold

at least a portion of the sample, and each of the anode compartment, cathode

compartment and at least one separation compartment includes means for an addition or

removal of a solution, a first compartment dimension, a second compartment dimension,

5   and a third compartment dimension. The first compartment dimension can be

substantially orthogonal to the direction of the electric field, the second compartment

dimension can be substantially orthogonal to the direction of the electric field and the first

compartment dimension. A ratio of the first compartment dimension and the second

compartment dimension defines an aspect ratio of the compartment, and the third

10   compartment dimension is preferably substantially parallel to the direction of the electric

field and substantially orthogonal to the first and second compartment dimensions. The

apparatus further includes an ion-permeable barrier positioned between each of the anode

compartment, the at least one separation compartment and the cathode compartment. The

ion-permeable barrier can be configured to prevent convective mixing therebetween. At

15   least a portion of at least one of the anode compartment, the cathode compartment and the

at least one separation compartment is made of an electrically insulating material having a

thermal conductivity greater than about 1 W/mK and a specific heat greater than about

100 J/kgK and the aspect ratio of at least one of the anode compartment, the cathode

compartment and the at least one separation compartment is less than one.

20   The present invention further provides for a method of altering a composition of a

sample by electrophoresis which includes providing an electrophoretic apparatus

according to the present invention. The method further includes selecting an ion-

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permeable barrier for use between the anode and cathode compartments, providing an

electrolyte to the anode compartment, providing an electrolyte to the cathode

compartment, providing at least a portion of a sample to at least one of the compartments,

creating an electrophoretic direct current between the anode and the cathode by applying

5   an electric potential between the anode and the cathode, and causing a transfer of at least

one part of at least one component of the sample across the ion-permeable barrier.

Alternatively, a method according to the present invention can include providing at least a

portion of a sample to at least one of the compartments of an apparatus according to the

present invention, providing at least one electrolyte to any of the compartments free of a

10   sample component, creating an electrophoretic direct current between the anode and the

cathode by applying an electric potential between the anode and the cathode, and causing

a transfer of at least one part of at least one component across an ion-permeable barrier.

Brief Description of the Drawings

        The accompanying drawings, which are incorporated herein and constitute part of

15   this specification, illustrate an embodiment of the invention, and, together with the

general description given above and the detailed description given below, serve to explain

features of the invention.

        FIGS. 1A and 1B are exploded top and plan cross-sectional views of a first

preferred embodiment of an electrophoresis apparatus according to the present invention;

20   FIG. 2A and 2B are exploded top and plan cross-sectional views of another

preferred embodiment of an electrophoresis apparatus according to the present invention;

        FIG. 3A and 3B are exploded top and plan cross-sectional views of another

preferred embodiment of an electrophoresis apparatus according to the present invention;

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FIG. 4A is a top view of a preferred embodiment of an electrode compartment or separation compartment for use in an electrophoresis apparatus according to the present invention;

FIG. 4B is a cross-sectional view of the compartment of FIG. 4A along line IVB-

5   IVB;

FIG. 4C is a plan view of the compartment of FIG. 4A along line IVC-IVC;

FIG. 5A is a top view of a preferred embodiment of an electrode compartment or separation compartment for use in the apparatus of FIG. 1;

FIG. 5B is a cross-sectional view of the compartment of FIG. 5A along line VB-

10   VB;

FIG. 5C is a plan view of the compartment of FIG. 5A;

FIG 6A is a cross-sectional view of a preferred embodiment of sealing means for use in another preferred embodiment of an electrophoresis apparatus according to the present invention;

15   FIG 6B is a plan view of the sealing means of FIG. 6A;

FIG. 7A is a preferred embodiment of a sealing means for use in the apparatus of FIG. 1;

FIG. 7B is a cross-sectional view of the sealing means of FIG. 7A along line VIIB-VIIB;

20   FIG. 7C is a plan view of the sealing means of FIG. 7A;

FIG. 8 is a graphic result of an imaging isoelectric focusing (ICIEF) analysis of a sample processed by an electrophoresis apparatus according to the present invention;

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FIG. 9 is a graphic result of an experiment for determining the isoelectric point of

a membrane using an electrophoresis apparatus according to the present invention;

FIG. 10 is a graphic result of an imaging isoelectric focusing (ICIEF) analysis of

fractions obtained from an egg-white sample using an electrophoresis apparatus according

5   to the present invention;

FIG. 11 is a graphic result of a polyacrylamide gel IEF separation of a sample

obtained using an electrophoresis apparatus according to the present invention;

FIG. 12 is another graphic result of another polyacrylamide gel IEF separation of

a sample obtained using an electrophoresis apparatus according to the present invention.

10   **Detailed Description**

Shown in FIGS. 1A, 1B, 2A, 2B, 3A, and 3B, are preferred embodiments of an

electrophoresis apparatus or device 10 for measuring, characterizing, and/or altering a

composition of a sample. Device 10 can be used to fractionate a biological sample so as

to add or remove at least a portion of a component from a sample solution. More

15   specifically, device 10 can be used in the pre-fractionation of protein samples for

proteomic analysis, the preparation of fractions for mass spectral analysis, bioactivity

testing, enzymatic analysis and other applications focused on the isolation of components.

In the embodiment shown in FIGS. 1A and 1B, apparatus 10 includes a first

element defining or forming anode compartment 14 and a second element defining or

20   forming cathode compartment 15, each of which can be individually inserted and axially

spaced apart within housing means 1 along a longitudinal axis A-A. Anode and cathode

compartments 14, 15 are each preferably configured to hold at least one electrolyte. In

addition, either anode or cathode compartments 14, 15 of apparatus 10 can be further

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configured to hold at least a portion of the sample to be altered. Each of anode and

cathode compartments **14, 15** have means for adding or removing a solution to or from its

respective compartment. As shown, anode and cathode compartments **14, 15** can be

preferably configured so as to have an opening from the top thereby making anode and

5   cathode compartment **14, 15** accessible for top loading or removal of a solution.

Alternatively, anode and cathode compartments can be configured with other structures or

alternatively located openings to provide access for adding or removing a solution from

the compartments. Preferably respectively disposed within anode and cathode

compartments **14, 15** are electrodes (not shown) acting as anode **30** (not shown) and

10   cathode **35** (not shown). Anode and cathode **30, 35** are axially spaced apart substantially

along longitudinal axis A-A by a distance **d** and can be further configured so as to provide

an electric field having a direction **E** substantially parallel to longitudinal axis A-A. The

electric field is applied for the purpose of performing the electrophoresis. Anode **30** and

cathode **35** can be connected to a power source (not shown), more preferably, anode **30**

15   and cathode **35** can be connected to a variable voltage source having a preferred voltage

ranging from about 10 V to about 5000 V, with a current preferably ranging from about

0.01 mA to about 1000 mA. It is to be understood that either compartment **14** or **15** can

act as the anode compartment and cathode compartment by connecting the appropriate

outlet of the power source to the electrode in the respective compartment functioning as

20   anode **30** and cathode **35**.

Preferably disposed between anode and cathode compartments **14, 15** and within

housing means **1** can be one or more separation elements defining or forming separation

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wells or compartments **40**. Although specifically shown in FIGS. 1A and 1B are first

separation compartment **22** and second separation compartment **23**, it is to be understood

that apparatus **10** can include as many separation compartments **40** as needed for a given

electrophoresis application. Each of separation compartments **40**, including first and

5   second separation compartments **22, 23** can be configured to hold at least one electrolyte

and can be preferably further configured to hold at least a portion of the sample to be

altered. Shown in FIGS. 2A and 2B is an alternative embodiment of apparatus **10'** having

a single separation compartment, and shown in FIGS. 3A and 3B is yet another

embodiment of apparatus **10''** having no separation compartment between anode and

10   cathode compartments **14** and **15**.

Housing means **1** orients and seals anode compartment **14**, cathode compartment

15 and where provided, separation compartment **40** such that compartments **14, 15** and **40**

are substantially aligned along longitudinal axis **A-A** so as to facilitate communication

therebetween in which components of the solution to be altered can migrate between

15   compartments **14, 15** and **40** under the influence of the electric field. In order to prevent

fluid loss from compartments **14, 15** and **40** to the environment, apparatus **10** can further

include sealing means **12**. Preferably, housing means **1** is configured so as to permit top

loading of anode, cathode, and separation compartments **14, 15, 40** and sealing means **12**

into housing means **1**.

20   Referring again to FIGS. 1A, 1B, 2A, 2B, 3A and 3B, sealing means **12** can be

disposed about each of anode and cathode compartments **14, 15** and about separation

compartments **40** where present. Each of sealing means **12** is preferably configured to

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contain ion-permeable barrier 18. Ion-permeable barrier 18 permits electrophoretic

migration of selected ions from one compartment 14, 15, 40 to another while substantially

restricting convective mixing of solutions contained in compartments 14, 15 and 40.

In order to facilitate the sealing action of sealing means 12, housing means 1 can

5   include axially opposed compression members 8, 9, preferably formed from an

electrically insulating, non-brittle, sufficiently rigid material, such as PVC material, that

can be axially displaced along longitudinal axis A-A to compress anode and cathode

compartments 14, 15, sealing means 12, ion-permeable barriers 18, and where present,

separation compartments 40. In addition, axial displacement of opposed compression

10   members 8, 9 facilitates removal and/or replacement of the individual anode, cathode and

separation compartments 14, 15, 40, sealing means 12 and ion-permeable barriers 18

from housing means 1. Compression members 8, 9 can directly act on axially opposed

end plates 16, 11 which are each preferably engaged with sealing means 12 to transmit

the compressive force to the assembled anode and cathode compartments 14, 15,

15   separation compartments 40, sealing means 12 and ion-permeable barriers 18.

Compression members 8, 9 can include a threaded rod and nut assembly 5 so as to axially

displace compression members 8, 9 along longitudinal axis A-A, however it is to be

understood that other means of linear displacement may be provided.

Preferably, at least a portion of housing means 1 is made from a material having a

20   thermal conductivity greater than about 1 W/mK, and a specific heat of greater than about

100 J/kgK, preferably greater than about 250 J/kgK, and especially greater than about 500

J/kgK. Referring to FIGS. 1A, 2A, and 3A housing means 1 can include insulating plate

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2 preferably formed from alumina (not shown) and base plate 3 preferably formed from

aluminum (not shown) and a cover (not shown). Preferably, anode, cathode and

separation compartments 14, 15, 40 and sealing means 12 and ion-permeable barriers 18

are located on insulating plate 2 (not shown). Insulating plate 2 can electrically isolate

5   base plate 3 from each of anode, cathode and separation compartments 14, 15, 40 and

sealing means 12 and ion-permeable barriers 18. Moreover, insulating plate 2 (made of

alumina) can act as a heat sink during the electrophoresis operation of apparatus 10, 10'

and 10." More preferably, the material forming housing 1 can be alumina, aluminum

nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon carbide,

10   ceramics, fused silica, quartz, glass or other ceramic materials or any combination

thereof. Moreover, base plate 3 (made of aluminum or stainless steel or other suitable

metal) can also act as a heat sink during the electrophoresis operation of apparatus 10, 10'

and 10".

       FIGS. 4A, 4B, and 4C and FIGS. 5A, 5B, and 5C are varying views of preferred

15   embodiments of removable anode compartment 14 and cathode compartment 15 of

apparatus 10, 10', 10" and of separation compartment 40 of apparatus 10, 10'. Anode,

cathode and separation compartments 14, 15 and 40 can be formed from alumina,

aluminum nitride, zirconia, zirconium nitride, boron nitride, silicon nitride, silicon

carbide, ceramics, fused silica, quartz, glass or other ceramic materials or any

20   combination thereof, so that heat generated during electrophoresis is dissipated to the

structural material of compartments 14, 15 and 40 to insure that the components in the

sample contained within compartments 14, 15 and 40 are not unduly heated. As a result,

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the need to provide external active (forced) cooling of either the electrolyte or the sample

solution can be greatly mitigated, and the electrophoretic power used to operate apparatus

10, 10' and **10''** can be increased. The undesirable surface characteristics of alumina,

zirconia, etc., (variable zeta potential, strong adsorptive binding of proteins) can be easily

5   modified by post-manufacturing surface treatment well known in the art, such as by

covalent or noncovalent binding of monomolecular layers or very thin films of protein-

binding inhibitors, e.g., hydrophilic organic materials or polymers, onto the surfaces that

are exposed to solutions. However, it should be understood that other electrical insulating

materials having a relatively high thermal conductivity and specific heat can be used as

10   well. More specifically, the material used to form any individual anode, cathode and

separation compartment **14, 15** and **40** have heat transfer properties including a thermal

conductivity higher than about 1 W/mK, preferably higher than about 10 W/mK,

especially higher than about 20 W/mK and having a specific heat higher than about 100

J/kgK, preferably higher than about 250 J/kgK, and especially higher than about 500

15   J/kgK.

Specifically shown in FIGS. 4A, 4B and 4C is an illustrative embodiment of

anode compartment **14** defined by first element of apparatus **10** as being a substantially

circular cylindrical disk-like member. However, other geometries of the first element

defining anode compartment **14** are possible, for example, as seen in the illustrative

20   embodiment of FIGS. 5A, 5B and 5C, showing an alternative embodiment of the first

element defining anode compartment **14'** as being substantially rectangular in cross-

section. Shown more specifically in each of FIG. 4A is a top view of the first element

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having an upper surface 17. Anode, cathode and separation compartments 14, 15 and 40

can be preferably formed by a grinding operation, but other techniques are possible, for

example, casting or molding. Alternatively, where a large number of anode, cathode and

separation compartments 14, 15, 40 are to be produced from alumina, compartment 14,

5   15 or 40 can be formed prior to firing of the alumina.

As seen in FIGS. 4A, 4B, 4C and 5A, 5B, and 5C, anode compartment 14, 14' is

preferably accessible through upper surface 17 so as to permit top loading of a sample or

electrolyte solution into compartment 14. Referring to FIGS. 4A, 4B, 4C and 5A, 5B and

5C, anode compartment 14, 14' is preferably defined by a width or first characteristic

10   dimension "a" a depth or second characteristic dimension "b" and a length or third

characteristic dimension "c". First dimension a and second dimension b define a

preferably substantially rectangular cross-section area 37 that is substantially orthogonal

to longitudinal axis A-A when, for example, anode compartment 14 is inserted in housing

1. However, other cross-sectional geometries are possible. Moreover, for example, when

15   anode compartment 14 is inserted in housing 1, first dimension a is preferably orthogonal

to longitudinal axis A-A or the direction E of the electric field, second dimension b is

preferably substantially orthogonal to both the first dimension a and the direction E of the

electric field and third dimension c is preferably substantially parallel to the direction E of

the electric field along longitudinal axis A-A. In addition, first and second dimensions a,

20   b define an aspect ratio of anode compartment 14 as a ratio of first dimension a to second

dimension b. First and second dimensions a and b are preferably selected such that the

aspect ratio of anode compartment 14 is less than one. Preferably, the aspect ratio is less

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than about ½, more preferably the aspect ratio is less than about 1/5, yet more preferably

the aspect ratio is less than about 1/10 and even more preferably the aspect ratio is less

than about 1/20.

It is to be understood that cathode compartment 15 and any number of separation

5   compartments 40 of apparatus 10, 10' or 10" can be independently similarly or variably

configured in a manner as described herein with respect to anode compartment 14. More

specifically, the aspect ratio of anode compartment 14 can be different from the aspect

ratio of cathode compartment 15 and/or separation compartments 40 by varying first and

second dimensions a, b of the respective compartments provided the aspect ratio of the

10   respective compartments remains less than one. In a preferred embodiment, separation

compartment 40 can be formed by grinding a 1.5 mm wide, 5 to 45 mm deep groove into

99.8% nonporous alumina blocks. Alternatively, grooves can be formed in alumina

blocks as thin as 0.25 mm and as thick as 2.5 mm.

Preferably first dimension a and third dimension c are minimized. Minimizing

15   first dimension a can in turn minimize the distance in the solution through which heat can

be conducted to the wall of anode compartment 14, cathode compartment 15 and/or

separation compartment 40. Minimizing third dimension c can mean that for a given

applied potential, the electric field strength, and consequently the electrophoretic

migration velocities of the components of the sample being processed are high, thus

20   reducing the required separation time. Moreover, by minimizing third dimension c, i.e.,

the migration distance in a particular compartment, the overall distance from anode

compartment 14 to cathode compartment 15 is minimized and therefore the separation

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time in processing the sample is once again further reduced. For example, all else being

equal, if one replaced a 9 mm I.D., 10 mm long cylindrical separation compartment (with

a volume of approximately 636 µl) by a 2 mm by 2 mm by 154 mm rectangular well with

a volume of approximately 616 µl (migration distance x width x height of the well), the

5   separation time would decrease about 25-fold (five times due to the reduced migration

distance and five times due to the five-fold higher electric field strength for a constant

applied potential). Additional benefits would accrue from the smaller temperature rise in

the separation well brought about by the smaller heat conduction distance (4.5 mm vs. 1

mm). Preferably, so as to facilitate minimization of first and third dimensions **a, c,**

10   apparatus **10, 10', 10''** is preferably configured such that compartments, **14, 15, 40,**

sealing means **12** and ion-permeable barriers **18** are substantially axially aligned within

housing means **1.**

Again referring to FIGS. 4A, 4B, 4C and 5A, 5B, and 5C, shown is anode

compartment **14, 14'** having first dimension **a.** First dimension **a** is preferably less than

15   about 5 mm, more preferably less than about 3 mm, and even more preferably less than

about 1 mm. The length of first dimension **a** can be varied, e.g., by the grinding operation

forming anode compartment **14.** For example, forming compartment **14** using a jig with

grinding wheels of different thickness allows for flexible changing of first dimension **a.**

Preferably, as shown, walls **38, 42** are parallel with respect to one another. Alternatively,

20   walls **38** and **42** can be tapered with respect to one another.

Third dimension **c** defines the migration distance of a component through anode

compartment **14,** cathode compartment **15** or separation compartment **40.** Referring to

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FIGS. 1A, 2A, and 3A, third dimension c of anode, cathode compartments **14, 15** and

where applicable, any one of separation compartments **40** in apparatus **10, 10'** and **10''**

can be either substantially equal or alternatively vary with respect to one another.

Preferably, third dimension c of separation compartments **40** is less than about half the

5   distance **d** between anode **30** and cathode **35**. More preferably, third dimension c of

separation compartment **40** is about less than 1/3 the distance **d** between anode **30** and

cathode **35**. An apparatus **10, 10'** having separation compartments with varying third

dimensions c so as to vary the migration distances in the compartments, can provide

flexibility in designing the shape of a pH gradient in the apparatus **10, 10'** and can further

10   accommodate major components in larger volumes and minor components in smaller

volumes. Moreover, the ability to have separation compartments **40** with varying third

dimensions c can also provide a means to concentrate desired components into smaller

volumes. Accordingly, where apparatus **10, 10'** can perform the electrophoresis process

with partially filled wells or compartments **14, 15** and/or **40**, samples of widely different

15   volumes can be handled in the same device.

Second dimension **b** or depth of the compartment permits the use of open (from

the top) compartments **14, 15** or **40**, and provides for variable (partial) filling of

compartments **14, 15** or **40** between zero and their respective full volume. There is no

theoretical limit to the magnitude of second dimension **b** of the separation compartment

20   orthogonal to the directions of both the electric field and first dimension of the

compartment. Second dimension **b** can be varied to increase or decrease the required

maximum reception volume of compartment **14, 15** or **40**, without degrading the

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separation speed or the thermal characteristics of apparatus **10, 10', 10''**. Second

dimension **b** can be varied by varying the dimensions of the material used to form

compartment **14, 15** or **40**, in conjunction with control of the grinding operation forming

compartment **14, 15** or **40**. Second dimension **b** can be as shallow as 5 mm and as deep

5   as 40 mm. Shown in FIGS. 4C and 5C, first dimension **a** is defined by the distance

between walls **38, 42** defining compartment **37**. Preferably as shown, walls **38, 42** are

parallel with respect to one another. Alternatively, walls **38, 42** can be tapered with

respect to one another.

First dimension **a**, second dimension **b** and third dimension **c** of each anode

10   compartment **14**, cathode compartment **15**, and separation compartment **40** defines a

reception volume for each to hold a volume of solution containing a sample component

and/or an electrolyte. Preferably, anode and cathode compartment **14, 15** and where

applicable, separation compartment **40**, of apparatus **10, 10', 10''** can receive a small

volume of a solution containing an electrolyte and/or a sample component, the volume

15   being less than about 5 ml, preferably less than about 2 ml, and more preferably between

about 0.5 ml to about 0.001 ml.

In one alternative embodiment (not shown) of apparatus **10** shown in FIGS. 1A

and 1B, apparatus **10** can include at least a first separation compartment **22** and at least a

second separation compartment **23** having a reception volume greater than the reception

20   volume of first separation compartment **22**. Preferably, the reception volume of anode

compartment **14** and the reception volume of cathode compartment **15** are greater than the

reception volumes of first and second separation compartments **22, 23**. In this

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embodiment, walls 38, 42 of anode compartment 14 and cathode compartment 15 are

preferably tapered relative to longitudinal axis A-A so as to produce a smooth transition

between anode and cathode compartments 14, 15 to first and second separation

compartments 22, 23.

5   Referring again to FIGS. 1A, 1B, 2A, 2B, 3A, and 3B, apparatus 10, 10' and 10''

can include sealing means 12 disposed about or in between each anode compartment 14,

cathode compartment 15 and where applicable, separation compartments 40 of apparatus

10, 10' and 10''. Shown in FIGS. 6A, 6B, 7A, 7B and 7C are preferred embodiments of

sealing means 12. In FIGS. 6A and 6B, sealing means 12 has a preferably substantially

10   cylindrical disk shape, preferably made of silicone. Alternatively, sealing means 12 can

be made from any water insoluble polymer, natural or synthetic, for example including,

but not limited to, polyethylene, polypropylene, polyisobutylene, polyalkylenes,

polyfluorocarbons, poly(dimethylsiloxane), poly(dialkylsiloxane),

poly(alkylarylsiloxane), poly(diarylsiloxane), poly(ether ether ketones) or a combination

15   thereof. Sealing means 12 further includes an opening 13 for providing an access through

which ions present in a solution in anode and cathode compartments 14, 15 and where

present, separation compartments 40 in apparatus 10, 10' and 10'' can migrate to and

access ion-permeable barrier 18. Opening 13 is preferably substantially rectangular and

includes a first characteristic dimension a' substantially corresponding to first dimension

20   a of anode compartment 14, cathode compartment 15 and where present, separation

compartment 40. Shown in FIGS. 1A, 2A, and 3A, preferably disposed between adjacent

sealing means 12 are ion-permeable barriers 18. Sealing means 12 can be configured to

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position, locate or contain ion-permeable barrier 18 within opening 13 of sealing means 12.

Other geometries of sealing means 12 are possible. A preferred alternative embodiment of sealing means 12 is shown in FIGS. 7A, 7B and 7C as 12'. Sealing means 12' is preferably formed from two silicone sheets joined together so as to form a pouch 19 for holding, containing and/or locating ion-permeable barrier 18. Pouch 19 can further effectively eliminate or significantly reduce the wicking action of the membrane forming ion-permeable barrier 18. Sealing members 12' and pouch 19 are preferably formed by adhesively joining two silicone sheets together around a removable pouch-defining shim (not shown) or by polymerizing the silicon material around a removable pouch-defining shim (not shown) to form pouch 19. The two silicone sheets used to form sealing means 12' are preferably pre-cut 0.5 mm or 0.25 mm thick silicone sheets. Alternatively, sealing means 12' can be made from any water insoluble polymer, natural or synthetic, for example including, but not limited to, polyethylene, polypropylene, polyisobutylene, polyalkylenes, polyfluorocarbons, poly(dimethylsiloxane), poly(dialkylsiloxane), poly(alkylarylsiloxane), poly(diarylsiloxane), poly(ether ether ketones) or a combination thereof. After sealing means 12' is formed, the shim is removed leaving pouch 19 for location of ion-permeable barrier 18. Pouch 19 is accessible from upper surface 21 of sealing means 12' so that ion-permeable barrier 18 can be loaded into pouch 19 from the top of sealing means 12.' Alternatively, ion-permeable barrier 18 can be completely encased in pouch 19 of sealing means 12', allowing it to communicate with its environment only through opening 13. Alternatively,

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sealing means **12'** can be cast or molded. Furthermore, the ability to create all seals at

once, rather than one by one, reduces the required minimum structural distance in the

direction of the electric field.

Sealing means **12'** includes an opening **13** for providing an access through which

5   ions in a solution contained in anode compartment **14**, cathode compartment **15** and

where present, separation compartment **40** can migrate to and access ion-permeable

barrier **18**. Opening **13** is preferably substantially rectangular and includes a first

characteristic dimension **a'** substantially corresponding to first dimension **a** of anode

compartment **14**, cathode compartment **15** and where present, separation compartment **40.**

10   Ion-permeable barrier **18** facilitates alteration by electrophoresis of a composition

of a sample contained in one or more of anode compartment **14**, cathode compartment **15**

and separation compartment **40** of apparatus **10, 10', 10''** of FIGS. 1A, 2A, and 3A,

respectively. Moreover, ion-permeable barrier **18** eliminates or mitigates convective

mixing of the contents of adjacent anode, cathode and separation compartments **14, 15**

15   and **40.** Ion-permeable barrier **18** can be a membrane having a defined pore size and pore

size distribution for size-based and charge-sign-based electrophoretic separation of the

sample components. Alternatively, ion-permeable barrier **18** can be an isoelectric

membrane suitable for isoelectric trapping (IET) separations. Ion-permeable barrier **18**

can also be configured so as to be essentially free of weekly acidic functional groups or

20   weakly basic functional groups or anionic functional groups or cationic functional groups.

Ion-permeable barrier **18** can also be configured to be an isoelectric membrane suitable

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for isoelectric trapping (IET) separations and have a defined pore size and pore size distribution.

For size-based separations, ion-permeable barrier 18 is preferably made from polyacrylamide and preferably has a nominal molecular mass cut-off from about 1 kDa to

5   1500 kDa. The molecular mass cut-off of the membrane material selected for ion-permeable barrier 18 will depend on the sample being processed and the type of components in the sample.

For IET-based separations, at least one ion-permeable barrier 18 can be an isoelectric membrane formed from any suitable material. Examples include, but are not

10   limited to, copolymers formed from acrylamide, bisacrylamide, acrylamido weak electrolytes and acrylamido strong electrolytes. Preferably, the membranes are thin or ultra-thin, having a thickness of about 2 mm or less, preferably about 1 mm or less, and especially about 0.2 mm or less. Where ion-permeable barrier 18 is an isoelectric membrane, barrier 18 is provided with a concentration of buffering species in the

15   membrane material. The isoelectric membrane forming ion-permeable barrier 18 does not have to be thick to provide adequate buffering capacity. As long as the isoelectric membrane forming ion-permeable barrier 18 can mitigate convective mixing between the contents of adjacent compartments 14, 15 and 40, the thinner the membrane, the shorter the distance the ampholytic components must travel. Therefore, thin isoelectric

20   membranes can lead to shorter separation times. Also, for all else being equal, the thinner the isoelectric membrane, the less potential drops across it, and thus the less power is consumed to effect the electrophoretic separation. Additionally, most solutions used for

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rehydration of the IPGIEF strips contain 0.1 - 1% carrier ampholytes. In IEF pre-
fractionation of proteomics samples, the fractions typically do not contain a single
isoelectric species with a single pI value, rather many components that cover a relatively
wide pI range (0.1 < pI < 2). This means that in the fractions, even at the end of the

5   separation, the carrier ampholyte and ampholytic sample molecules are typically not in
their isoelectric state, but are protonated and deprotonated by each other. This also means
that in these fractions the ionic strength is higher than at the end of an IET separation in
which pure, single components are produced in a compartment. If, due to the improved
heat dissipation performance of electrophoresis apparatus 10 one could add, in a

10   sufficiently high concentration, carrier ampholytes or auxiliary isoelectric buffers to the
sample prior to electrophoresis, one could significantly increase the ionic strength in the
respective fractions. This would improve protein solubility and increase the total amount
of material that can be loaded or processed in the given volume of the system.

        The characteristics of ion-permeable barrier 18 used depend on the sample and the

15   type of separation or treatment contemplated. Within a single apparatus 10, 10' or 10'',
ion-permeable barriers 18 used may each be variably configured in a manner described
herein to suit the electrophoresis application as needed. Ion-permeable barriers 18 or
membranes can be purchased for use in the apparatus or made by the user prior to
carrying out the desired electrophoresis run.

20   Referring again to FIGS. 1A, 2A and 3A, to assemble electrophoresis apparatus
10, 10' and 10'', anode and cathode compartments 14, 15 and where provided, separation
compartments (wells) 40, sealing means 12 and ion-permeable barriers 18 preferably

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installed in pouches **19** can be placed into housing means **1** preferably from above. Once

compartments **14, 15, 40**, sealing means **12** and ion-permeable barriers **18** are in place,

wing nuts **5** are turned gently until they become finger tight over compression members

8,9 to create the seals. Compartments **14, 15, 40** are then filled with deionized water for a

5   brief leak test. Once the system passes the leak test, anode and cathode compartments **14,**

15 are filled with the respective anolyte and catholyte solutions, separation compartments

40 are preferably filled with the sample and where provided, an electrolyte, anode **30** and

cathode **35** are respectively lowered into anode and cathode compartments **14, 15** and the

electrophoretic potential is applied. In the case of an IET separation, the IET separation

10 can be carried out using either constant potential, constant current or constant power

input. Once the IET separation is complete (as indicated by the time course of the

potential or the current), power is turned off and the contents of compartments **14, 15, 40**

are removed for subsequent analysis or use.

In one exemplary assembled embodiment of an electrophoresis apparatus **10**,

15 unfilled polycarbonate, for example, LEXAN® available from BOEDEKER PLASTICS,

TX, is used to form housing means **1** and a ½ inch diameter borosilicate glass rod is used

to form five separation compartments **40**, each having a holding volume of 50 μl. Ion-

permeable barriers **18** are formed from isoelectric membranes which are installed

between sealing means **12** formed from silicone disks which reduce solution loss from

20 membrane wicking. Such an assembled apparatus **10** could be used for the separation of

low molecular weight pI markers and proteins and for UV-active carrier ampholyte-based

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membrane characterization. Using a surface treatment with a hydrophilic polymer on

sealing means 12 can further reduce leaking problems and mitigate electroosmotic flow.

In another exemplary embodiment of an electrophoresis apparatus 10, housing

means 1 is preferably constructed from LEXAN® and seven separation compartments 40

5   are preferably formed from a 3/4 inch diameter borosilicate glass rod. Each separation

compartment 40 defines a receiving volume of 150 μl. Ion-permeable barriers 18 are

preferably isoelectric membranes installed in sealing means 12 including circular silicone

pouches 19 that completely prevent liquid loss by wicking. Such an assembled apparatus

10 can be used for the separation of low molecular weight pI markers and proteins and for

1 0   UV-active carrier ampholyte-based membrane characterization.

In another exemplary embodiment of an electrophoresis apparatus 10, housing

means 1 is preferably constructed from LEXAN® and six separation compartments 40

are preferably formed from rectangular ½ x ¼ x 1 inch, nonporous, 99.8% alumina

blocks. Each separation compartment 40 defines a second dimension b of about 5 mm.

1 5   Such an assembled apparatus 10 can be used for IET desalting, the separation of low

molecular weight pI markers and proteins and for UV-active carrier ampholyte-based

membrane characterization. Using a surface treatment with a hydrophilic polymer on

sealing means 12 can further reduce leaking problems and practically eliminate

electroosmotic flow.

2 0   In another exemplary embodiment of an electrophoresis apparatus 10, housing

means 1 is preferably constructed from LEXAN® and ten separation compartments 40

are preferably formed from rectangular, ½ x ¼ x 1 inch, nonporous, 99.8% alumina

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blocks, each having a second dimension **b** being about 18 mm deep. Such an assembled

apparatus **10** can be used for IET desalting, the separation of low molecular weight pI

markers and proteins, for UV-active carrier ampholyte-based membrane characterization,

and for the selection of the appropriate isoelectric membranes for larger scale membrane-

5   based IET separations.

In yet another exemplary embodiment of an electrophoresis apparatus **10**, housing

means **1** is preferably constructed from LEXAN® and twenty separation compartments

40 are preferably formed from rectangular, 2 x 35 x 55 mm, nonporous alumina blocks,

each defining second dimension **b** as being about 40 mm deep. Such an assembled

10   apparatus **10** can be used for IET desalting, the separation of low molecular weight pI

markers and proteins, for UV-active carrier ampholyte-based membrane characterization

and for the selection of the appropriate isoelectric membranes for larger scale membrane-

based IET separations.

The method of altering a composition of a sample by electrophoresis using an

15   apparatus **10, 10'** or **10''** includes selecting an ion-permeable barrier **18** for use between

the anode and cathode compartments based upon the given application. Upon providing

anode and cathode compartment **14, 15** with the requisite electrolyte, the sample can be

added to one or more of compartments **14, 15** and **40**. Alternatively, using an apparatus

10, 10' or **10''**, a sample can be added to one or more compartments **14, 15,** or **40** and an

20   electrolyte can be added to any compartment **14, 15,** or **40** that does not contain the

sample. Alternatively, using apparatus **10, 10',** or **10''** both a sample and an electrolyte

can be added to one or more of compartments **14, 15,** or **40** and an electrolyte can be

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added to any compartment **14, 15,** or **40** that does not contain the sample. Subsequently,

an electrophoretic direct current between the anode and the cathode can be provided by

applying an electric potential between the anode and the cathode so as to cause a part of a

component of the sample being processed to transfer across an ion-permeable barrier **18.**

5   In a first preferred method of processing a sample using an electrophoresis

apparatus **10, 10'** or **10'',** all ion-permeable barriers **18** inter-disposed in housing means **1**

are preferably anti-convective isoelectric barriers. Selecting ion-permeable barriers **18** of

this type produces fractions with predetermined pI ranges, *i.e.*, the system is operated in

pure IET mode. The pI cuts can be as narrow or as broad as desired, depending on the

10   characteristics of the sample and the objective of the electrophoretic separation, *i.e.*,

prefractionation, selective component removal and/or enrichment of a component of the

sample being processed. Fractionation can be achieved in the presence or absence of

carrier ampholytes and auxiliary isoelectric buffers. This method of processing is

especially flexible when compartments **14, 15** and **40** are variable within a single

15   apparatus **10, 10', 10''** with respect to first characteristic dimension a.

In the second or alternative method of processing a sample using an

electrophoresis apparatus **10, 10'** or **10'',** ion-permeable barriers **18** adjacent to anode

compartment **14** and cathode compartment **15** are preferably anti-convective, isoelectric

barriers. All other ion-permeable barriers **18** of apparatus **10, 10'** are preferably anti-

20   convective, ion-permeable, non-isoelectric membranes. The fractions produced in the

anode, cathode and/or separation compartments **14, 15, 40** still have distinct pI ranges.

However, the respective pI ranges are not known a-priori, rather they depend on the

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composition of the solution, *i.e.*, the relative amount of the carrier ampholytes, if used, the

isoelectric auxiliary agent(s), if used, and the analytes (pure autofocusing mode). The

advantage of this method or processing is that it allows for the production of fractions

with pI ranges for which no isoelectric membranes are available. The drawback of this

5   second method can be that the pI range boundaries associated with individual

compartments **40** cannot be defined by the user ahead of the time. This second method of

processing a sample is especially flexible when apparatus **10, 10'** includes a large number

of separation compartments **40**, each with a very small third characteristic dimension c.

In another or third method of processing a sample using an electrophoresis

10   apparatus **10** having at least two separation compartments **40**, ion-permeable barriers **18**

adjacent to anode compartment **14** and cathode compartment **15** are preferably anti-

convective isoelectric barriers, at least one ion-permeable barrier **18** inter-disposed

between separation compartments **40** is preferably an anti-convective, isoelectric barrier,

and at least one other ion-permeable barrier **18** is preferably an anti-convective, non-

15   isoelectric barrier. Using this alternative method, the fractions produced in anode,

cathode and/or separation compartments **14, 15, 40** also have distinct pI ranges: for some

of them the pI range depends on the pI values of the isoelectric membranes delimiting the

individual separation compartments **40**, for others it depends on the composition of the

solution, i.e., the relative amount of the carrier ampholytes, if used, the isoelectric

20   auxiliary agent(s), if used, and the analytes (mixed IET – autofocusing mode). This third

or alternative method of processing a sample is advantageous when the pI boundaries for

a major sample component are not known exactly, but one still would like to isolate

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minor components with slightly lower and slightly higher pI values than the pI value of a

major component. The drawback of the method is that the exact pI range boundaries of

all the fractions cannot be defined by the user ahead of time. The third operation mode

also benefits from the use of a relatively large number of separation compartments **40**

5   having a very small third characteristic dimension c.

In yet another or fourth method of processing a sample using an electrophoresis

apparatus **10, 10'**, ion-permeable barriers **18** adjacent to anode compartment **14** and

cathode compartment **15** are preferably anti-convective, isoelectric barriers. The

solutions in anode, cathode and separation compartments **14, 15, 40** can contain one or

10   more isoelectric auxiliary agents. Additionally, at least one of ion-permeable barriers **18**

of apparatus **10, 10'** is preferably an anti-convective barriers having a characteristic, size-

dependent permeability. This alternative method of processing a sample allows for a

size-based fractionation of components, especially when the amounts of sample

components are relatively small compared to that of the isoelectric auxiliary agent(s)

15   retained in the system by isoelectric trapping.

In another alternative or fifth method of processing a sample using an

electrophoresis apparatus **10, 10', 10''** all ion-permeable barriers **18** are anti-convective

and have a characteristic size-dependent permeability. At least one of anode, cathode and

separation compartments **14, 15** and **40** can contain a solution of one or more isoelectric

20   auxiliary agents. This method can be used for a rapid desalting of the sample or a size-

based or charge-sign-based separation of its components. In a preferred method of

desalting using the fifth method of processing a sample in apparatus **10, 10'**, the smaller

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the number of separation compartments **40** provided, the faster the desalting, though the use of at least one separation compartment **40** adjacent to each of anode and cathode compartments **14, 15** might reduce the extent of protic shock for the sample components.

In yet another alternative or sixth method of processing a sample using an

5   electrophoresis apparatus **10**, (known as a matrix deployment method), a plurality of separation compartments **40** and inter-disposed isoelectric ion-permeable barriers **18** ranging between a low and a high pI are provided, e.g., twelve separation compartments **40** and ten inter-disposed isoelectric ion-permeable barriers **18** ranging between a pI of 2 to a pI of 12 are provided, where the pI of each successive ion-permeable barrier **18**

10   increases by 1.0. A complex biological sample, for example, a sample intended for proteomic analysis, is loaded into one or more of the ten separation compartments of first apparatus **10**. Anode and cathode compartments **14, 15** of first apparatus **10** are filled with an anolyte and catholyte, respectively. In this preferred method of use of electrophoresis apparatus **10**, the fractions produced in compartments **40** define the ten

15   rows of a separation matrix.

After performing an IET separation for 10 to 30 minutes using first apparatus **10**, the content of each separation compartment **40** is transferred, preferably simultaneously, into ten separate apparatuses **10**, each having an anode compartment, a cathode compartment and ten separation compartments **40**. The ten apparatuses **10** in the second

20   set of apparatuses define ten columns of the separation matrix. Accordingly, separation compartments **40** present in this second set of apparatuses **10** define the elements of the separation matrix. Ion-permeable barriers **18** adjacent to anode and cathode

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compartments **14, 15** in apparatus **10** defining the columns of the separation matrix have

the same pI values as ion-permeable barriers **18** inter-disposed between the respective

separation compartments **40** of first apparatus **10** defining the rows of the separation

matrix. Thus, e.g., ion-permeable barrier **18** between the anode compartment and the first

5   separation compartment of apparatus **10** defining the first column of the separation matrix

has a pI of 2, and ion-permeable barrier **18** between the cathode compartment and the last

separation compartment of apparatus **10** defining the first column of the separation matrix

has a pI of 3; ion-permeable barrier **18** between the anode compartment and the first

separation compartment of apparatus **10** defining the second column of the separation

10   matrix has a pI of 3, and ion-permeable barrier **18** between the cathode compartment and

the last separation compartment of apparatus **10** defining the second column of the

separation matrix has a pI of 4; ion-permeable barrier **18** between the anode compartment

and the first separation compartment of apparatus **10** defining the third column of the

separation matrix has a pI of 4, and ion-permeable barrier **18** between the cathode

15   compartment and the first separation compartment of apparatus **10** defining the third

column of the separation matrix has a pI of 5; etc. In each apparatus **10** defining the

columns of the separation matrix, separation compartments **40** are isolated from each

other by anti-convective, non-isoelectric ion-permeable barriers **18**. Thus, a temporally

stable pH gradient is formed during the second electrophoretic separation across

20   separation compartments **40** in each apparatus **10** defining the columns of the separation

matrix, with the shape of the respective pH gradients depending on the relative amounts

of the carrier ampholytes, where used, the auxiliary isoelectric buffers, where used, and

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the sample constituents. Thus, separation compartments 40 in the first and second sets of

apparatuses 10 define the elements of the separation matrix (10 x 10 = 100), and each

respective separation compartment 40 contains fractions with a pI range of about 0.1.

The resulting fractions can then be directly analyzed by mass spectroscopy, used for

5   further research or digested and analyzed by mass spectrometry as common in proteomics

to identify the constituent proteins.

        If needed, the fractions can be subdivided further, preferably in another

electrophoresis apparatus 10 wherein ion-permeable barriers 18 having a characteristic,

size-dependent permeability are used in a manner substantially similar to the sixth method

10   of processing a sample as described above. The resulting fractions can then be directly

analyzed by mass spectroscopy, used for further research or digested and analyzed by

mass spectrometry as common in proteomics to identify the constituent proteins. If

needed, the respective digests can also be subjected to a subsequent IET separation in

another apparatus 10 to provide fractions containing peptides with similar acidities. Such

15   fractions are preferred for mass spectrometric analysis. This matrix operation mode

provides a purely liquid-vein alternative 2DE-MS method for an analysis of the

constituents of a complex, proteomic sample.

        In an alternative method to the matrix deployment method, the content of each

separation compartment 40 from the first IET separation is first stored, then sequentially

20   transferred, ten times, into the ten separation compartments (wells) of the same,

sequentially used electrophoresis apparatus 10, and the IET analysis defining the columns

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of the separation matrix is accomplished over a longer period of time, requiring only a single apparatus 10.

In yet another alternative embodiment of the matrix deployment method described above, a first apparatus 10 having twenty-two separation compartments 40 is assembled using twenty inter-disposed isoelectric ion-permeable barriers 18 having pI values ranging between a pI of 2 to a pI of 12, where the pI of each successive ion-permeable barrier 18 increases by 0.5. A complex biological sample, for example, a sample intended for proteomic analysis, is loaded into one or more of the twenty separation compartments of first apparatus 10. Anode and cathode compartments 14, 15 of first apparatus 10 are filled with an anolyte and catholyte, respectively. In this preferred method of use of electrophoresis apparatus 10, the fractions produced in compartments 40 define the twenty rows of the separation matrix.

After performing an IET separation for 10 to 30 minutes using first apparatus 10, the content of each separation compartment 40 is transferred, preferably simultaneously, into twenty separate apparatuses 10, each having an anode compartment, a cathode compartment and twenty separation compartments 40. This second set of electrophoretic devices, comprised of twenty apparatuses 10, defines the columns of the separation matrix. Accordingly, separation compartments 40 present in this second set of apparatuses 10 define the elements of the separation matrix. Ion-permeable barriers 18 adjacent to anode and cathode compartments 14, 15 in apparatus 10 defining the columns of the separation matrix have the same pI values as ion-permeable barriers 18 inter-disposed between the respective separation compartments of first apparatus 10 defining

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the rows of the separation matrix. Thus, e.g., ion-permeable barrier **18** between the anode

compartment and the first separation compartment of apparatus **10** defining the first

column of the separation matrix has a pI of 2, and ion-permeable barrier **18** between the

cathode compartment and the last separation compartment of apparatus **10** defining the

5   first column of the separation matrix has a pI of 2.5; ion-permeable barrier **18** between the

anode compartment and the first separation compartment of apparatus **10** defining the

second column of the separation matrix has a pI of 2.5, and ion-permeable barrier **18**

between the cathode compartment and the last separation compartment of apparatus **10**

defining the second column of the separation matrix has a pI of 3; ion-permeable barrier

10   **18** between the anode compartment and the first separation compartment of apparatus **10**

defining the third column of the separation matrix has a pI of 3.0, and ion-permeable

barrier **18** between the cathode compartment and the first separation compartment of

apparatus **10** defining the third column of the separation matrix has a pI of 3.5; etc. In

each apparatus **10** defining the columns of the separation matrix, separation

15   compartments **40** are isolated from each other by anti-convective, non-isoelectric ion-

permeable barriers **18**. Thus, a temporally stable pH gradient is formed during the second

electrophoretic separation across separation compartments **40** in each apparatus **10**

defining the columns of the separation matrix, with the shape of the respective pH

gradients depending on the relative amounts of the carrier ampholytes, where used, the

20   auxiliary isoelectric buffers, where used, and the sample constituents. Thus, separation

compartments **40** in the first and second sets of apparatuses **10** define the elements of the

separation matrix (20 x 20 = 400), and each respective separation compartment **40**

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contains fractions with a pI range of about 0.025. The resulting fractions can then be directly analyzed by mass spectroscopy, used for further research or digested and analyzed by mass spectrometry as common in proteomics to identify the constituent proteins.

5   If needed, the fractions can be subdivided further, preferably in another electrophoresis apparatus 10 wherein ion-permeable barriers 18 having a characteristic, size-dependent permeability are used in a manner substantially similar to the method of processing a sample as described above. Due to the fine pI resolution, the number of size-based fractions required might be relatively low (e.g., 4 to 6). The resulting fractions

10   can then be directly analyzed by mass spectroscopy, used for further research or digested and analyzed by mass spectrometry as common in proteomics to identify the constituent proteins. If needed, the respective digests can also be subjected to a subsequent IET separation in another apparatus 10 to provide fractions containing peptides with similar · acidities. Such fractions are preferred for mass spectrometric analysis. This high

15   resolution matrix operation mode can provide a purely liquid-vein alternative to the 2DE-MS analysis of the constituents of a complex, proteomic sample and is believed to be just as (or more) powerful as the currently used prefractionation-2DE-MS methods, while being more suitable for robotics-based automation.

         Another or seventh method of processing a sample includes using an

20   electrophoresis apparatus 10 in which dilute samples or fractions can be concentrated by IET. A preferred apparatus 10 is assembled using separation compartments 40, more specifically, a first separation compartment 22 and at least a second separation

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compartment **23** smaller that the first separation compartment **22,** each compartment

disposed between anode compartment **14** and cathode compartment **15.** Larger separation

compartment **22** is preferably located adjacent to anode or cathode compartment **14, 15**

(or both, if two larger separation compartments **22** are used). Preferably, anode and

5   cathode compartments **14, 15** are each relatively large as compared to first and at least

second compartments **22, 23.** Walls **37, 42** of first separation compartment **22** are

preferably tapered, producing a smooth transition between anode and cathode

compartments **14, 15** having preferably wider first dimensions **a** and second separation

compartment **23** having preferably narrower first dimension **a.** To provide adequate

10   potential drop across separation compartments **22, 23,** at least one isoelectric buffer is

added to the sample to be fractionated. The pI value of the added isoelectric buffer is

selected such that the isoelectric buffer is trapped in first separation compartment **22,**

between isoelectric ion-permeable barriers **18** separating anode and cathode

compartments **14, 15** and first separation compartment **22,** and isoelectric ion-permeable

15   barrier **18** separating the large volume wells and at least one second smaller separation

compartment **23.** In another preferred embodiment, simultaneous concentration and

fractionation can be achieved using a plurality of separation compartments **40** separated

by isoelectric or non-isoelectric ion-permeable barriers **18.**

EXAMPLES

20   **Example 1: Fractionation of low molecular weight pI markers**

An electrophoresis apparatus was assembled using six alumina elements that each

contain a 40 x 2 x 2.5 mm compartment. The anode, cathode and four separation

compartments were separated by five ion-permeable barriers made from isoelectric

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membranes respectively having pI values of: pI = 2, pI = 3, pI = 5, pI = 6.5, and pI = 9.5. The anode compartment was filled with 60 mM methanesulfonic acid, and the cathode compartment was filled with a mixture of 20 mM lysine and 20 mM arginine. The separation compartment delimited by ion-permeable barriers of pI = 2 and pI = 3

5   contained 50 mM IDA. Nominal 200 μl aliquots of a sample containing 2% Pharmalyte 3 < pI < 10 carrier ampholytes and three pI markers: nicotinic acid (pI = 3.2), 4-hydroxy-2-(morpholinomethylene)-benzoic acid (pI = 5.8) and epinephrine (pI = 9.2) were loaded into each of the separation compartments of the apparatus. The power supply was operated at a constant power of 4 W for 14 min, yielding an initial potential of 213 V,

10 final potential of 575 V, initial current of 16 mA and final current of 7 mA. The separation took a total of 121 Vh.

The content of each well was analyzed by the iCE280 ICIEF system (Convergent Bioscience, Toronto, Canada) before the IET separation and after the IET separation. The respective volume changes, the component peak areas and their ratios are set out in Table

15 1.

Table 1

Well	pI range	Vol change (μl)	Marker	Area$_{init}$	Area$_{final}$	Ratio
1	pI < 2	+5				
2	2 < pI < 3	0				'
3	2 < pI < 3	-5	Nic	16490	44630	2.71
4	5 < pI < 6.5	+5	Morph	4412	12625	2.86

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5	6.5< pI < 9.5	-5	Epi	20966	57278	2.73
6	9.5 < pI	0				

The results of an ICIEF run are shown in FIG. 8. Clearly, the UV-absorbing pI

markers were completely moved in 14 min into the wells limited by the appropriate

isoelectric membranes.

5   **Example 2: Characterization of the pI of an isoelectric separation membrane**

An electrophoresis apparatus was assembled using four alumina elements that

each contain a 40 x 2 x 2.5 mm separation compartment. The anode, cathode and two

separation compartments were isolated by three ion-permeable barriers made from

isoelectric membranes, the first of which had a pI value of 2 , the second one was the

10   membrane to be tested, and the third one was a membrane with a pI value of 11.5. The

anode compartment was filled with 60 mM methanesulfonic acid and the cathode

compartment was filled with 60 mM NaOH. Nominal 200 µl aliquots of a sample

containing 2% Pharmalyte 3 < pI < 10 carrier ampholytes and 0.1% UV active carrier

ampholytes were loaded into the two separation compartments. The power supply was

15   operated at a constant power of 6 W for 15 min. After IET, the contents of the well

adjacent to the anode compartment and the cathode compartment were analyzed by ICIEF

using the iCE280 unit. The results are shown in Figure 9. Clearly, the carrier ampholytes

were separated into two fractions indicating that the pI of the isoelectric membrane to be

tested was 7.5.

20   **Example 3: Fractionation of an egg-white sample**

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An electrophoresis apparatus was assembled using five alumina elements that each contain a 40 x 2 x 2.5 mm alumina compartment. The anode, cathode and three separation compartments were isolated by ion-permeable barriers made from isoelectric membranes respectively having pI values of: pI = 4; pI = 5.6, pI = 8.5 and a pI = 12. The

5   anode compartment was filled with 50 mM IDA and the cathode compartment was filled with 60 mM NaOH. Nominal 200 µl aliquots of filtered egg white dissolved in 2% Pharmalyte 3 < pI < 10 carrier ampholytes were loaded into each of the three separation compartments. The power was operated at a constant potential of 500 V for 18 min, yielding a final current of 4 mA. After IET separation, the content of each

10 compartment was analyzed by the iCE280 ICIEF system.

The results are shown in FIG. 10. The top panel is the ICIEF result for the pI markers, the second panel is the egg white feed sample mixed with the pI markers, the third panel is for the 4 < pI < 5.6 fraction, the fourth panel is for the 5.6 < pI < 8.5 fraction, and the fifth panel is for the 8.5 < pI < 12 fraction. The major proteins in each

15 fraction (ovalbumin, ovotransferrin and lysozyme) reach their final destination well in as short a separation time as 18 min.

Example 4: Binary fractionation of a calf liver lysate sample

An electrophoresis apparatus was assembled using four alumina elements that each contain a 40 x 2 x 2.5 mm compartment. The anode, cathode and two separation

20 compartments were separated by three ion-permeable barriers respectively having pI values of: pI = 5, pI = 6.5 and pI = 9.5. The anode compartment was filled with 60 mM CH_3SO_3H and the cathode compartment was filled with a mixture of 20 mM lysine and 20 mM arginine. Nominal 200 µl aliquots of 0.5 mg/ml calf liver lysate (7 M urea, 2 M

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thiourea, 4% CHAPS, 3% 3 < pI < 10 Pharmalyte carrier ampholytes) were loaded into

each of the three separation compartments. The power supply was operated at a constant

power of 4 W for 15 min, yielding a final current of 5 mA. After IET separation, the

content of each compartment was analyzed by IEF using 3 < pI < 10 IEF gels

5   (Invitrogen). Results of the separation are shown in FIG. 11. There was a very sharp cut

between the two protein fractions indicating that the IET separation was complete in as

little as 15 min.

Example 5: Fractionation of a calf liver lysate sample

An electrophoresis apparatus was assembled using five alumina elements that

10   each contain a 40 x 2 x 2.5 mm compartment. The anode, cathode and three separation

compartments were separated by four ion-permeable barriers made from isoelectric

membranes respectively having pI values of: pI = 3, pI = 5, pI = 6.5 and pI = 9.5. The

anode compartment was filled with 60 mM CH_3SO_3H and the cathode compartment was

filled with a mixture of 20 mM lysine and 20 mM arginine. Nominal 200 µl aliquots of

15   0.5 mg/ml calf liver lysate (7 M urea, 2 M thiourea, 4% CHAPS, 3% 3 < pI < 10

Pharmalyte carrier ampholytes) were loaded into a single separation compartment

delimited by ion-permeable barriers of pI = 3 and pI = 5. The power supply was

operated at a constant power of 4 W for 15 min, yielding a final current of 5 mA. After

IET separation, the content of each well was analyzed by IEF using 3 < pI < 10 IEF gels

20   (Invitrogen). Results of the separation are shown in Figure 12. There was a very sharp

cut between the three protein fractions indicating that the IET separation was complete in

as little as 25 min.

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The electrophoresis apparatus described herein addresses many of the

disadvantages of currently used isoelectric pre-fractionation apparatuses and methods

such as the inability to tolerate high electric power loads, the need for active cooling,

slow separation speeds, inconvenient system set-up and sample handling, and relatively

5   large sample volumes that cannot be varied easily. The apparatus described herein may

be used to separate varying volumes of complex samples into multiple fractions, with

direct recovery of the fractions for subsequent analytical or biological characterization, in

10 to 30 min, using 5 to 10 W power, without active (forced) external cooling.

While the present invention has been disclosed with reference to certain

10   embodiments, numerous modifications, alterations, and changes to the described

embodiments are possible without departing from the sphere and scope of the present

invention, as defined in the appended claims. Accordingly, it is intended that the present

invention not be limited to the described embodiments, but that it have the full scope

defined by the language of the following claims, and equivalents thereof.